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CHEMICAL ABSTRACTS

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**EP 0 080 304 B1**

## Description

This invention relates to a process for stabilizing an oxidase and a composition containing the stabilized oxidase for use in clinical chemical examinations.

Recently, clinical chemical examinations have been developed remarkably as examination techniques for diagnosis of diseases and watching of the course of treatments. Particularly, the progress and spread of automatic chemical analysis equipments makes the examinations not only rapid and accurate but also more important in hospital examinations. The automatic chemical analysis equipments require new type measuring reagents. That is, the reagents should complete the reaction in a short time at a mild temperature such as about 37°C in order to apply to such equipments. Thus, enzymatic measuring methods using enzymes have been developed. At present, almost all blood components can be determined quantitatively by enzymatic methods.

In such enzymatic methods, it becomes important to stabilize a reagent solution containing an enzyme for a long period of time. For example, measurement of neutral fat (triglyceride) is an important test item, since it can be an important indication for finding abnormal lipids metabolism, diagnosis of diseases such as diabetes mellitus and judgement of treatment course. For measuring triglyceride, glycerol-3-phosphate oxidase is used. But glycerol-3-phosphate oxidase is not good in stability, particularly in an aqueous solution.

It is also known that oxidases are generally unstable. Stabilization of oxidases other than glycerol-3-phosphate oxidase is also desirable. Examples of such oxidases are choline oxidase, glucose oxidase, etc.

Main constituent of enzymes is proteins. Enzymes show special enzymatic actions depending on space structures of these proteins. But the space structures vary by influences of various factors and thus enzymes lose activities. Therefore, it is necessary to stabilize the enzymes.

There are proposed many processes for stabilizing enzymes. One process is to add a substrate or a co-enzyme to an enzyme to be stabilized. In proteins of enzymes, there are one or more local portions having strain which is unstable from the viewpoint of energy. Such portions often become active portions of enzymes. When a substrate or a coenzyme is bonded to such portions, such portions are stabilized from the viewpoint of energy, which results in stabilizing the enzymes. Another process is to add a SH protecting reagent to enzymes. When an enzyme has an active portion having a SH group, it is effective to add a SH protecting reagent such as mercaptoethanol, dithiothreitol or the like to such an enzyme. Further, non-specific stabilizers are sometimes used. For example, inert proteins such as albumin, glycerol, lactose, etc. are sometimes effective.

But, in the case of oxidases such as glycerol-3-phosphate oxidase, choline oxidase, glucose oxidase, etc., such stabilizing methods or stabilizers as mentioned above are not effective at all.

EP—A—0 009 222 discloses stabilisation of certain dehydrogenases such as lactate dehydrogenase but has no general disclosure as to the stabilisation of oxidases and requires very specific conditions. Similarly Chemical Abstracts 93:68683a discloses stabilisation of sarcosine oxidase but is not otherwise generally applicable. There is no indication of methods of stabilising the oxidases of the present invention. Other oxidases such as cholesterol oxidase are not stabilised by the technique of the present invention.

Objects of this invention is to provide a process for stabilizing oxidases, a composition containing such a stabilized oxidase for use in clinical chemical examinations.

This invention provides a process for stabilizing an oxidase selected from the group consisting of glycerol-3-phosphate oxidase, choline oxidase and glucose oxidase which comprises adding an acidic amino acid or a salt thereof to said oxidase.

This invention also provides a composition for use in clinical chemical examinations comprising an oxidase selected from the group consisting of glycerol-3-phosphate oxidase, choline oxidase and glucose oxidase, and an acidic amino acid or a salt thereof.

The invention also provides a diagnostic test kit for the determination of hydrogen peroxide which comprises

a) a container having a composition comprising  
a buffer solution,  
an oxidase selected from the group consisting of glycerol-3-phosphate oxidase, choline oxidase and glucose oxidase, and

an acidic amino acid or a salt thereof, and  
b) a container having a composition comprising  
a color producing reagent, and  
a solvent for the color producing reagent.

In the attached drawings, Figs. 1 to 4 show relationships between residual activity of glycerol-3-phosphate oxidase and elapsed time with or without addition of a salt of acidic amino acid, Fig. 5 shows a relationship between residual activity of choline oxidase and elapsed time with or without addition of a salt of acidic amino acid, Fig. 6 shows a relationship between residual activity of glucose oxidase and elapsed time with or without addition of a salt of acidic amino acid, and Fig. 7 shows a relationship between residual activity of cholesterol oxidase and elapsed time with or without addition of a salt of acidic amino acid (comparison).

It is a very important and surprising thing that the addition of an acidic amino acid (aminodicarboxylic

acid) or a salt thereof to an oxidase selected from the group consisting of glycerol-3-phosphate oxidase, choline oxidase and glucose oxidase makes the oxidase stabilize without giving undesirable influences on the measurement in clinical chemical examinations to be conducted afterwards.

The stabilizing agent usable in this invention is an acidic amino acid or a salt thereof, preferably a buffer solution-soluble salt thereof. Such a stabilizing agent is preferably represented by the formula:



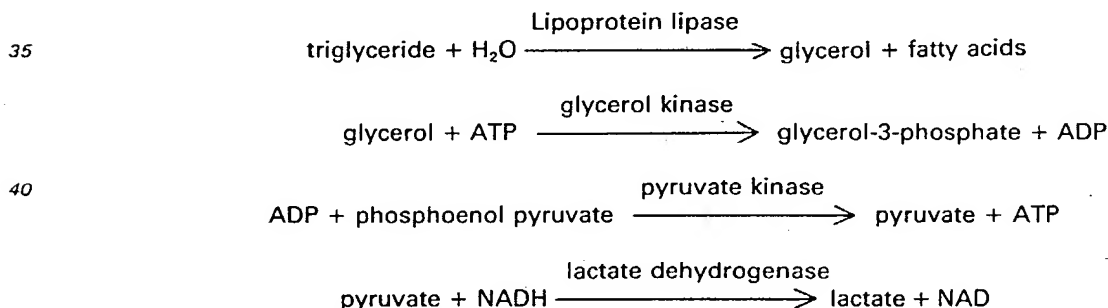
wherein A is a lower alkylene group preferably having 1 to 5 carbon atoms; and Y and Y' are independently hydrogen, a  $\text{NH}_4$  group or an alkali metal. Preferable examples of the acidic amino acid of the formula (I) are glutamic acid, aspartic acid, mono- or diammonium salt of glutamic acid or aspartic acid, mon- or dialkali metal salts of glutamic or aspartic acid such as sodium glutamate, sodium aspartate, potassium glutamate, potassium aspartate, etc. The use of alkali metal salt of glutamic or aspartic acid is preferable considering solubility. It is possible to use other acidic amino acids such as  $\alpha$ -aminoadipic acid, and the like.

The acidic amino acid or a salt thereof is added in an amount of 1 to 5% by weight to the aqueous solution containing an oxidase to be stabilized. The oxidase content in the aqueous solution changes depending on the kinds of oxidases to be stabilized but usually 1 to 20 units/ml (U/ml) for glycerol-3-phosphate oxidase and choline oxidase and 1 to 100 U/ml for glucose oxidase. If the amount is too much, the stability of coloring in the clinical chemical examination is damaged. Usually, about 3% by weight is more preferable.

Oxidases to be stabilized by the invention are glycerol-3-phosphate oxidase, choline oxidase and glucose oxidase. Although these oxidases belong to flavin enzymes, the stabilizing process of this invention is only applicable to limited members of flavin enzymes. For example, the process of this invention is not effective for cholesterol oxidase which belongs to flavin enzymes.

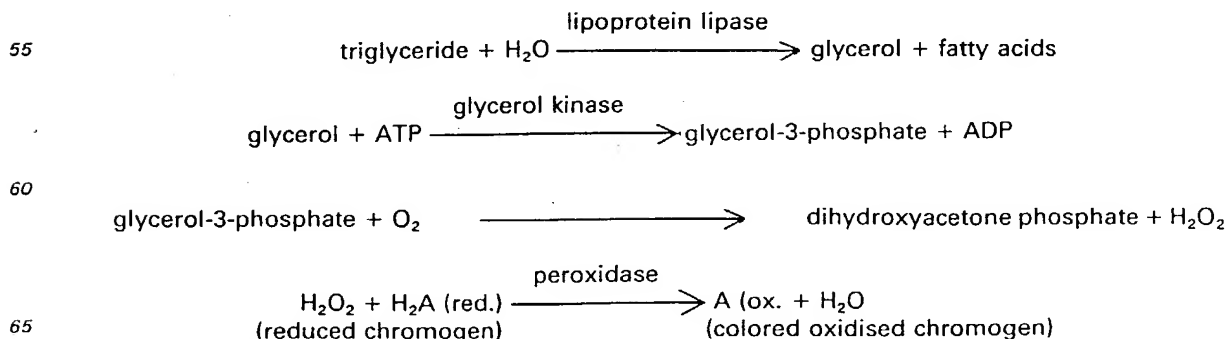
Glycerol-3-phosphate oxidase is an oxidase which can be obtained via culture and extraction from strains of aerococcus or streptococcus, but it is unstable particularly in the form an aqueous solution.

Glycerol-3-phosphate oxidase is used, for example, for measuring triglyceride. Triglyceride (neutral fat) was measured by using enzymes as follows:



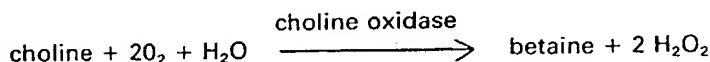
That is, by measuring a decrease in absorbance at 340 nm at which NADH shows a specific adsorption, the content of triglyceride in a sample can be obtained. According to this method, since the absorbance occurs at 340 nm, which belongs to ultraviolet region, it is necessary to use an ultraviolet spectrophotometer and further, when serum is used as a sample, specimen blank has a great influence on the measurement.

But, recently, a colorimetric method using wavelengths in visible light region is developed in contrast to the above-mentioned method. Such a method uses glycerol-3-phosphate oxidase and can be represented by the following equations:



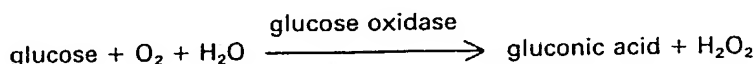
In the above-mentioned reaction equations, when an indicator which produces a color in a visible light region is used, it becomes possible to employ a colorimetric method in the visible light region while overcoming disadvantages of the old process mentioned above. Thus, to stabilize an aqueous solution of glycerol-3-phosphate oxidase becomes very important.

Choline oxidase is an oxidase which can be obtained from strains of *arthrobacter* or *alcaligenes*. Choline oxidase accelerates the following reaction:



Therefore, in a system wherein choline is present or choline is produced, it becomes possible to employ a colorimetric method in the visible light region wherein  $\text{H}_2\text{O}_2$  produced is measured. Further, as applications of choline oxidase to quantitative methods of living samples, it is possible to measure activity of choline esterase and to measure quantitatively the amount of phospholipids wherein phospholipase D is combined and liberated choline is measured.

Glucose oxidase is an oxidase which can be obtained from strains of *aspergillus* and accelerates the following reaction:



Glucose oxidase has wide applications for measuring living samples, for example, quantitative determination of glucose in a body fluid, measuring of activity of amylase, etc. In measuring activity of amylase using starch as a substrate, glucose obtained by decomposition of starch via glucoamylase is measured quantitatively.

A stabilized reagent composition containing glycerol-3-phosphate oxidase for measuring glycerol-3-phosphate will now be discussed. A reagent solution containing glycerol-3-phosphate oxidase, peroxidase and an indicator, said reagent solution per se being known for measuring glycerol-3-phosphate quantitatively, can be stabilized by adding an acidic amino acid or a salt thereof such as alkali metal salt of aminodicarboxylic acid thereto. Thus, a stabilized reagent composition for use in clinical applications can be obtained, said composition containing glycerol-3-phosphate oxidase, an alkali metal salt of aminodicarboxylic acid as a stabilizer and a buffer solution, and if necessary an indicator for colorimetric determination and one or more conventional additives.

In the same manner as mentioned above, stabilized reagent compositions comprising choline oxidase or glucose oxidase, a stabilizer of the formula (I), and a buffer solution, and if necessary one or more conventional additives such as an indicator for colorimetric determination, and the like can be obtained.

In the case of measuring the content of triglyceride in a living sample such as serum, etc., there can be used a reagent solution for measurement prepared by dissolving lipoprotein lipase, glycerol kinase, ATP, glycerol-3-phosphate oxidase, peroxidase and an indicator in a suitable buffer solution such as tris buffer solution.

In the reaction using such a reagent composition, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is produced for glycerol-3-phosphate by the action of glycerol-3-phosphate oxidase. When an oxidizable color producing indicator is present in such a case, said indicator produces the color by  $\text{H}_2\text{O}_2$  in the presence of peroxidase.

As the oxidizable color producing indicator, there can be used o- or p-phenylenediamine, dianisidine, and the like indicators alone, or a combination indicator such as 4-aminoantipyrine and phenol, a halophenol or an aniline derivative, etc.

As the buffer solution, there can be used any ones which can maintain the desired pH. Examples of such buffer solutions are a tris buffer solution, Good buffer solution, phosphate buffer solution, and the like. The pH preferable for the reaction is near neutral value and pH 7.5 is more preferable.

Further, as is clear from the reaction equations mentioned above, any substances such as triglyceride, glycerol, ATP, and glycerol-3-phosphate can be measured. But the most important reaction step among these reaction equations is the reaction using glycerol-3-phosphate oxidase.

Glycerol-3-phosphate oxidase obtained from *aerococcus viridans* or *streptococcus faecalis* by a conventional process is very unstable in an aqueous solution and can only be used for a few hours after dissolving.

But according to the stabilizing process of this invention, an aqueous solution of glycerol-3-phosphate oxidase can be stored at  $5^\circ\text{C}$  for one week stably by adding an aminodicarboxylic acid (acidic amino acid) or a salt thereof and can be used for measurement during such a period. (See Figs. 1 to 4.)

In the same manner as mentioned above, an aqueous solution of choline oxidase can be stabilized and can be used for measurement after stored at  $20^\circ\text{C}$  for one week, and an aqueous solution of glucose oxidase can be used for measurement after stored at  $40^\circ\text{C}$  for 3 days. (See Figs. 5 and 6.)

This invention is illustrated by way of the following Examples.

## Example 1

## (1) Determination of Glycerol-3-phosphate

*Reagent Solution*

A reagent solution for measuring glycerol-3-phosphate was prepared by dissolving the following ingredients in 0.05 M tris buffer solution (pH 7.5):

	Glycerol-3-phosphate oxidase	5 U/ml
	Peroxidase	2.5 U/ml
10	p-Chlorophenol	0.07% by weight
	4-Aminoantipyrine	0.15 mg/ml
15	Sodium glutamate	3.0% by weight

*Measuring Operations*

A sample (e.g. a biological fluid such as serum) in an amount of 0.02 ml was added to 3.0 ml of the reagent solution for measurement obtained by the above formulation. After mixing well, color was produced by warming at 37°C for 10 minutes. On the other hand, using 0.02 ml of distilled water, a reagent blank was prepared in the same manner as mentioned above.

Absorbances at 505 nm were measured using the reagent blank as control. Absorbances of standard solutions prepared by dissolving certain amounts of glycerol-3-phosphate in various concentrations were also measured in the same manner as mentioned above and the content of glycerol-3-phosphate in the sample was obtained by proportion calculations of the absorbances obtained.

## (2) Stabilization of Aqueous Solution of Glycerol-3-phosphate Oxidase

A 0.05 M tris buffer solution (pH 7.5) dissolving 0.07% by weight of p-chlorophenol, 5 U/ml of glycerol-3-phosphate oxidase and 3.0% by weight of sodium glutamate was maintained at 5°C and residual activity of glycerol-3-phosphate oxidase was measured with the lapse of time. For comparison, the same composition as mentioned above except for not containing sodium glutamate was also prepared and measured in the same manner as mentioned above.

The results are as shown in Fig. 1.

When 3.0% by weight of sodium aspartate was used in place of sodium glutamate, the results are as shown in Fig. 2, which also shows the results of Fig. 1.

When sodium glutamate was dissolved in a 0.05 M tris buffer solution (pH 7.5) in various concentrations (5%, 3%, 1%, and 0% by weight) together with 5 U/ml of glycerol-3-phosphate oxidase and kept at 20°C, the residual activity of glycerol-3-phosphate oxidase with the lapse of time was shown in Fig. 3.

When sodium aspartate was dissolved in a 0.05 M tris buffer solution (pH 7.5) in various concentrations (3%, 1% and 0% by weight) together with 5 U/ml of glycerol-3-phosphate oxidase and kept at 20°C, the residual activity of glycerol-3-phosphate oxidase with the lapse of time was as shown in Fig. 4.

## Example 2

## Determination of Triglyceride in a Living Sample

*Reagent Solution*

A reagent solution for measuring triglyceride was prepared by dissolving the following ingredients in 0.05 M tris buffer solution (pH 7.5):

	Lipoprotein lipase	40 U/ml
50	Glycerol kinase	2.5 U/ml
	Glycerol-3-phosphate oxidase	5 U/ml
55	Peroxidase	2.5 U/ml
	Magnesium acetate	5 mmoles/l
	p-Chlorophenol	0.07% by weight
60	4-Aminoantipyrine	0.15 mg/ml
	ATP	1 mg/ml
65	Sodium glutamate	3% by weight

*Measuring Operations*

A living sample of biological fluid (e.g. serum) in an amount of 0.02 ml was added to 3.0 ml of the reagent solution for measuring triglyceride and mixed well. Color was produced by warming at 37°C for 10 minutes. On the other hand, using 0.02 ml of distilled water, a reagent blank was prepared in the same manner as mentioned above.

Absorbances at 505 nm were measured using the reagent blank as control. Absorbances of standard solutions prepared by dissolving certain amounts of glycerol in various concentrations were also measured. The glycerol content was obtained by proportion calculations, after which the triglyceride content was calculated by converting to the triglyceride amount.

## Example 3

## (1) Determination of Choline

*Reagent Solution*

A reagent solution for measuring choline was prepared by dissolving the following ingredients in a 0.05 M phosphate buffer solution (pH 7.6):

Choline oxidase	2.5 U/ml
Peroxidase	1.0 U/ml
4-Aminoantipyrine	0.015% byweight
Phenol	0.1% by weight
Sodium glutamate	3.0% by weight

*Measuring Operations*

A sample (e.g. a biological fluid such as serum) in an amount of 0.02 ml was added to 3.0 ml of the reagent solution for measuring choline and mixed well. Color was produced by warming at 37°C for 10 minutes. On the other hand, using 0.02 ml of distilled water, a reagent blank was prepared in the same manner as mentioned above.

Absorbances at 505 nm were measured using the reagent blank as control. Absorbances of standard solutions prepared by dissolving certain amounts of choline chloride in various concentrations were also measured. The choline content in the sample was obtained by proportion calculations of the absorbances obtained.

## (2) Stabilization of Aqueous Solution of Choline Oxidase

A 0.05 M phosphate buffer solution (pH 7.6) dissolving 2.5 U/ml of choline oxidase and 0.1% by weight of phenol together with sodium glutamate (5%, 3%, 1% and 0% by weight) or sodium aspartate (3%, 1% and 0% by weight) was maintained at 20°C. The residual activity of choline oxidase was measured with the lapse of time and shown in Fig. 5.

## Example 4

## Measurement of Activity of Choline Esterase in Living Sample

*Reagent Solution*

## (A) Substrate enzyme solution

A substrate enzyme solution was prepared by dissolving the following ingredients in a 0.02 M phosphate buffer solution (pH 7.6):

Choline oxidase	2.5 U/ml
Peroxidase	1.0 U/ml
4-Aminoantipyrine	0.015% byweight
Choline benzoyl chloride	0.015% byweight
Phenol	0.2% by weight
Sodium glutamate	3.0% by weight

## (B) Reaction stopper solution

A reaction stopper solution was prepared by dissolving 100 mg of neostigmine methylsulfate in 100 ml of distilled water.

*Measuring Operations*

In a test tube, 2.0 ml of the substrate enzyme solution was placed and armed at 37°C for 3 minutes in a constant temperature water bath. Subsequently, 0.02 ml of a sample (e.g. a biological fluid such as serum) was added to the test tube and mixed well. After warming at 37°C for just 5 minutes, 2.0 ml of the reaction stopper solution was added thereto. On the other hand, using 0.02 ml of distilled water, a reagent blank was prepared in the same manner as mentioned above.

Absorbances at 505 nm were measured using the reagent blank as control. Absorbances of serum having known activity values were measured in same manner as mentioned above and activity value of choline esterase in the sample was obtained by proportion calculations of the absorbances obtained.

## Example 5

## (1) Determination of Glucose

*Reagent Solution*

A reagent solution for measuring glucose was prepared by dissolving the following ingredients in a 0.2 M phosphate buffer (pH 7.4):

Glucose oxidase	30 U/ml
Mutarotase	0.1 U/ml
Peroxidase	1.0 U/ml
4-Aminoantipyrine	0.1% by weight
Phenol	0.1% by weight
Sodium aspartate	5.0% by weight

*Measuring Operations*

A sample (e.g. a biological fluid such as serum) in an amount of 0.2 ml was added to 3.0 ml of the reagent solution for measuring glucose and mixed well. Color was produced by warming at 37°C for 10 minutes. On the other hand, using 0.02 ml of distilled water, a reagent blank was prepared in the same manner as mentioned above.

Absorbances at 505 nm were measured using the reagent blank as control. Absorbances of standard solutions prepared by dissolving certain amounts of glucose in various concentration were also measured. The glucose content in the sample was obtained by proportion calculations of the absorbances obtained.

## (2) Stabilization of Aqueous Solution of Glucose Oxide

A 0.2 M phosphate buffer solution (pH 7.4) dissolving 30 U/ml of glucose oxidase and 0.1% by weight of phenol together with sodium glutamate (5%, 3%, 1% and 0% by weight) of sodium aspartate (5%, 3%, 1% and 0% by weight) was maintained at 40°C. The residual activity of glucose oxidase was measured with the lapse of time and shown in Fig. 6.

## Comparative Example 1

## (1) Determination of Cholesterol

*Reagent Solution*

A reagent solution of measuring cholesterol was prepared by dissolving the following ingredients in a 0.1 M phosphate buffer solution (pH 7.0):

Cholesterol oxidase	0.2 U/ml
Peroxidase	1.0 U/ml
4-Aminoantipyrine	0.015% byweight
Phenol	0.1% by weight

*Measuring Operations*

A sample (e.g. a biological fluid such as serum) in an amount of 0.02 ml was added to 3.0 ml of the reagent solution for measuring cholesterol and mixed well. Color was produced by warming at 37°C for 10 minutes. On the other hand, using 0.2 ml of distilled water, a reagent blank was prepared in the same manner as mentioned above.

Absorbances at 505 nm were measured using the reagent blank as control. Absorbances of standard solutions prepared by dissolving certain amounts of cholesterol in isopropyl alcohol in various concentrations were also measured. The cholesterol content in the sample was obtained by proportion

calculations of the absorbances obtained. The same results were also obtained when sodium aspartate or sodium glutamate (3% by weight) was added to the reagent solution.

## (2) Stabilization of Aqueous Solution of Cholesterol Oxidase

5 A 0.1 M phosphate buffer solution (pH 7.0) dissolving 0.2 U/ml of cholesterol oxidase and 0.1% by weight of phenol together with sodium glutamate (3% by weight) or sodium aspartate (3% by weight) or without sodium glutamate or sodium aspartate was maintained at 20°C. The residual activity of cholesterol oxidase was measured with the lapse of time and shown in Fig. 7.

As shown in Fig. 7, there is shown no stabilizing effect of sodium aspartate or sodium glutamate in the  
10 case of aqueous solution of cholesterol oxidase.

## Claims

1. A process for stabilizing an oxidase which comprises adding an acidic amino acid or a salt thereof to  
15 an oxidase selected from the group consisting of glycerol-3-phosphate oxidase, choline oxidase and glucose oxidase.

2. A process according to Claim 1, wherein the oxidase is glycerol-3-phosphate oxidase.

3. A process according to Claim 1, wherein the oxidase is choline oxidase.

4. A process according to Claim 1, wherein the acidic amino acid or a salt thereof is a buffer solution-  
20 soluble salt of acidic amino acid.

5. A process according to Claim 1, wherein the acidic amino acid or a salt thereof is represented by the formula:



wherein A is a lower alkylene group having 1 to 5 carbon atoms; and Y and Y' are independently hydrogen,  
30 a NH<sub>4</sub> group or an alkali metal.

6. A process according to Claim 1, wherein the acidic amino acid or a salt thereof is sodium glutamate.

7. A process according to Claim 1, wherein the acidic amino acid or a salt thereof is sodium aspartate.

8. A process according to Claim 1, wherein the acidic amino acid or a salt thereof is added in an amount  
of 1 to 5% by weight to an aqueous solution containing the oxidase to be stabilized.

35 9. A stabilized composition comprising

a buffer solution,

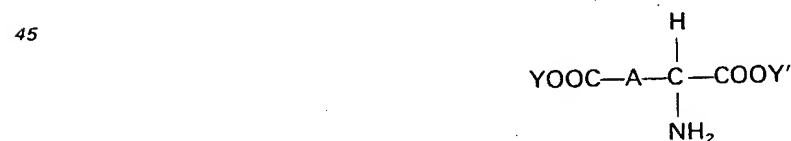
an oxidase selected from the group consisting of glycerol-3-phosphate oxidase, choline oxidase and  
glucose oxidase, and

an acidic amino acid or a salt thereof.

40 10. A composition according to Claim 9, wherein the oxidase is glycerol-3-phosphate oxidase.

11. A composition according to Claim 9, wherein the oxidase is choline oxidase.

12. A stabilized composition according to Claim 9, wherein the acidic amino acid or a salt thereof is  
represented by the formula:



50 wherein A is a lower alkylene group having 1 to 5 carbon atoms; and Y and Y' are independently hydrogen, a NH<sub>4</sub> group or an alkali metal.

13. A composition according to Claim 9, which further comprises a color producing reagent.

14. A composition according to Claim 9, wherein the acidic amino acid or a salt thereof is sodium  
55 glutamate.

15. A composition according to Claim 9, wherein the acidic amino acid or a salt thereof is sodium aspartate.

16. A diagnostic test kit for the determination of hydrogen peroxide which comprises

a) a container having a composition comprising

60 a buffer solution,

an oxidase selected from the group consisting of glycerol-3-phosphate oxidase, choline oxidase and  
glucose oxidase, and

an acidic amino acid or a salt thereof, and

b) a container having a composition comprising

65 a color producing reagent, and



a solvent for the color producing reagent.

17. Use of an amino acid stabilised oxidase composition comprising glycerol-3-phosphate oxidase, a buffer solution,

an acidic amino acid or a salt thereof,

peroxidase, and

4-aminoantipyrine,

for quantitatively determining the glycerol-3-phosphate content in a biological fluid.

18. A use according to Claim 17, wherein the acidic amino acid or a salt thereof is an alkali metal salt of acidic amino acid.

19. A use according to Claim 18, wherein the alkali metal salt of acidic amino acid is sodium aspartate or sodium glutamate.

20. Use of an amino acid stabilised oxidase composition comprising

a lipase,

glycerol kinase,

glycerol-3-phosphate oxidase,

peroxidase,

4-aminoantipyrine,

adenosine triphosphate

an acidic amino acid or a salt thereof, and

a buffer solution,

for quantitatively determining the triglyceride content in a biological fluid.

21. A use according to Claim 20, wherein the acidic amino acid or a salt thereof is an alkali metal salt of acidic amino acid.

22. A use according to Claim 21, wherein the alkali metal salt of acidic amino acid is sodium aspartate or sodium glutamate.

23. Use of an amino acid stabilised oxidase composition comprising choline oxidase,

peroxidase,

4-aminoantipyrine,

an acidic amino acid or a salt thereof, and

a buffer solution,

for quantitatively determining the choline content in a biological fluid.

24. A use according to Claim 23, wherein the acidic amino acid or a salt thereof is an alkali metal salt of acidic amino acid.

25. A use according to Claim 24, wherein the alkali metal salt of acidic amino acid is sodium aspartate or sodium glutamate.

26. Use of an amino acid stabilised oxidase composition comprising

choline oxidase,

peroxidase,

4-aminoantipyrine,

choline benzyl chloride,

an acidic amino acid or a salt thereof, and

a buffer solution,

for measuring activity of choline esterase in a biological fluid.

27. A use according to Claim 26, wherein the acidic amino acid or a salt thereof is an alkali metal salt of acidic amino acid.

28. A use according to Claim 27, wherein the alkali metal salt of acidic amino acid is sodium aspartate or sodium glutamate.

29. Use of an amino acid stabilised oxidase composition comprising glucose oxidase,

peroxidase,

mutarotase,

4-aminoantipyrine,

an acidic amino acid or a salt thereof, and

a buffer solution,

for quantitatively determining the glucose content in a biological fluid.

30. A use according to Claim 29, wherein the acidic amino acid or a salt thereof is an alkali metal salt of acidic amino acid.

31. A use according to Claim 30, wherein the alkali metal salt of acidic amino acid is sodium aspartate or sodium glutamate.

## Patentsprüche

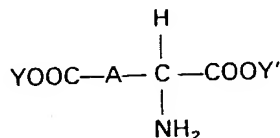
1. Verfahren zum Stabilisieren einer Oxidase, in dem eine saure Aminosäure oder ein Salz derselben einer Oxidase, zugesetzt wird, die aus der Gruppe ausgewählt ist, die aus der Glycerin-3-phosphatoxidase, der Cholinoxidase und der Glukoseoxidase besteht.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die Oxidase Glycerin-3-phosphatoxidase ist.

3. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die Oxidase Cholinoxidase ist.

4. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben ein in einer Pufferlösung lösliches Salz einer sauren Aminosäure ist.

5. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben durch die Formel



15 dargestellt wird, in der A eine niedere Alkylengruppe mit 1 bis 5 Kohlenstoffatomen ist und Y und Y' unabhängig voneinander Wasserstoff, eine  $\text{NH}_4$ -Gruppe oder ein Alkalimetall sind.

6. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben Natriumglutamat ist.

7. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben Natriumspartat ist.

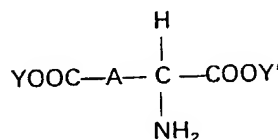
8. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben in einer Menge von 1 bis 5 Gew.% einer wässrigen Lösung zugesetzt wird, die die zu stabilisierende Oxidase enthält.

9. Stabilisierte Zusammensetzung mit einer Pufferlösung, einer Oxidase, die aus der Gruppe ausgewählt ist, die aus der Glycerin-3-phosphatoxidase, der Cholinoxidase und der Glucoseoxidase besteht, und einer sauren Aminosäure oder einem Salz derselben.

10. Zusammensetzung nach Anspruch 9, dadurch gekennzeichnet, daß die Oxidase Glycerin-3-phosphatoxidase ist.

11. Zusammensetzung nach Anspruch 9, dadurch gekennzeichnet, daß die Oxidase Cholinoxidase ist.

12. Stabilisierte Oxidase nach Anspruch 9, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben durch die Formel



40 dargestellt wird, in der A eine niedere Alkylengruppe mit 1 bis 5 Kohlenstoffatomen ist und Y und Y' unabhängig voneinander Wasserstoff, eine  $\text{NH}_4$ -Gruppe oder ein Alkalimetall sind.

13. Zusammensetzung nach Anspruch 9, die außerdem ein farberzeugendes Reagens enthält.

14. Zusammensetzung nach Anspruch 9, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben Natriumglutamat ist.

15. Zusammensetzung nach Anspruch 9, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben Natriumspartat ist.

16. Diagnostischer Prüfsatz zur Bestimmung von Wasserstoffperoxid, mit

a) einem Behälter, der eine Zusammensetzung enthält, die eine Pufferlösung enthält, ferner eine Oxidase, die aus der Gruppe ausgewählt ist, die aus der Glycerin-3-phosphatoxidase, der Cholinoxidase

und der Glucoseoxidase besteht, und

eine saure Aminosäure oder ein Salz derselben, und

b) einem Behälter, der eine Zusammensetzung enthält, die ein farberzeugendes Reagens und ein Lösungsmittel für das farberzeugende Reagens enthält.

17. Die Verwendung einer aminosäurestabilisierten Oxidasezusammensetzung, die Glycerin-3-phosphatoxidase,

eine Pufferlösung,

eine Aminosäure oder ein Salz derselben, Peroxidase und

4-Aminoantipyrin

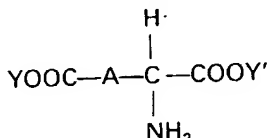
enthält, zur quantitativen Bestimmung des Glycerin-3-phosphatgehaltes eines biologischen Mediums.

18. Verwendung nach Anspruch 18, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben ein Alkalimetallsalz einer sauren Aminosäure ist.

19. Verwendung nach Anspruch 18, dadurch gekennzeichnet, daß das Alkalimetallsalz der sauren Aminosäure Natriumaspargat oder Natriumglutamat ist.
20. Die Verwendung einer aminosäurestabilisierten Oxidasezusammensetzung mit einer Lipase,  
 5 Glycerinkinase,  
 Glycerin-3-phosphatoxidase,  
 Peroxidase,  
 4-Aminoantipyrin,  
 Adenosintriphosphat,  
 10 einer sauren Aminosäure oder ein Salz derselben und  
 einer Pufferlösung  
 zur quantitativen Bestimmung des Triglyceridgehalts eines biologischen Fluids.
21. Verwendung nach Anspruch 20, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben ein Alkalimetallsalz einer sauren Aminosäure ist.
- 15 22. Verwendung nach Anspruch 21, dadurch gekennzeichnet, daß das Alkalimetallsalz der sauren Aminosäure Natriumaspargat oder Natriumglutamat ist.
23. Die Verwendung einer aminosäurestabilisierten Oxidasezusammensetzung mit Cholinoxidase,  
 Peroxidase,  
 20 4-Aminooxidase,  
 einer sauren Aminosäure oder einem Salz derselben und  
 einer Pufferlösung  
 zur quantitativen Bestimmung des Cholingehalts eines biologischen Fluids.
24. Verwendung nach Anspruch 23, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz  
 25 derselben ein Alkalimetallsalz einer sauren Aminosäure ist.
25. Verwendung nach Anspruch 24, dadurch gekennzeichnet, daß das Alkalimetallsalz der sauren Aminosäure Natriumaspargat oder Natriumglutamat ist.
26. Die Verwendung einer aminosäurestabilisierten Oxidasezusammensetzung mit Cholinoxidase,  
 30 Peroxidase,  
 4-Aminoantipyrin,  
 Cholinpenzoylchlorid,  
 einer sauren Aminosäure oder einem Salz derselben und  
 einer Pufferlösung  
 35 zur Messung der Aktivität der Cholinesterase in einem biologischen Fluid.
27. Verwendung nach Anspruch 26, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben ein Alkalimetallsalz einer sauren Aminosäure ist.
28. Verwendung nach Anspruch 27, dadurch gekennzeichnet, daß das Alkalimetallsalz der sauren Aminosäure Natriumaspargat oder Natriumglutamat ist.
- 40 29. Verwendung einer aminosäurestabilisierten Oxidasezusammensetzung mit Glucoseoxidase,  
 Peroxidase,  
 Mutarotase,  
 4-Aminoantipyrin,  
 45 einer sauren Aminosäure oder einem Salz derselben und  
 einer Pufferlösung  
 zur quantitativen Bestimmung des Glucosegehalts eines biologischen Fluids.
30. Verwendung nach Anspruch 19, dadurch gekennzeichnet, daß die saure Aminosäure oder das Salz derselben ein Alkalimetallsalz einer sauren Aminosäure ist.
- 50 31. Verwendung nach Anspruch 30, dadurch gekennzeichnet, daß das Alkalimetallsalz der sauren Aminosäure Natriumaspargat oder Natriumglutamat ist.

#### Revendications

- 55 1. Procédé de stabilisation d'une oxydase, qui comprend l'addition d'un aminoacide acide ou d'un de ses sels à une oxydase choisie parmi la glycérol-3-phosphate oxydase, la choline oxydase et la glucose oxydase.
2. Procédé suivant la revendication 1, dans lequel l'oxydase est la glycérol-3-phosphate oxydase.
- 60 3. Procédé suivant la revendication 1, dans lequel l'oxydase est la choline oxydase.
4. Procédé suivant la revendication 1, dans lequel l'acide amino ou l'un de ses sels est un sel soluble dans une solution tampon d'un aminoacide acide.
5. Procédé suivant la revendication 1, dans lequel l'acide amino ou un de ses sels est représenté par la formule:
- 65



5 dans laquelle A est un groupe alkylène inférieur ayant 1 à 5 atomes, de carbone; et Y et Y' sont indépendamment l'hydrogène, un groupe NH<sub>4</sub> ou un métal alcalin.

6. Procédé suivant la revendication 1, dans lequel l'acide aminé ou un de ses sels est le glutamate de sodium.

7. Procédé suivant la revendication 1, dans lequel l'acide aminé ou un de ses sels est l'aspartate de sodium.

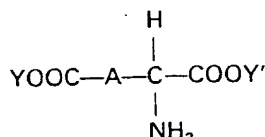
8. Procédé suivant la revendication 1, dans lequel l'acide aminé ou l'un de ses sels est ajouté dans une quantité de 1 à 5 % en poids à une solution aqueuse contenant l'oxydase à stabiliser.

9. Une composition stabilisée comprenant une solution tampon, une oxydase choisie parmi la glycérol-3-phosphate oxydase, la choline, oxydase et la glucose oxydase, et un acide aminé ou un de ses sels.

10. Composition suivant la revendication 9, dans laquelle l'oxydase est la glycérol-3-phosphate oxydase.

11. Composition suivant la revendication 9, dans laquelle l'oxydase est la choline oxydase.

12. Composition stabilisée suivant la revendication 9, dans laquelle l'acide aminé ou un de ses sels est représenté par la formule:



30 dans laquelle A est un groupe alkylène inférieur ayant 1 à 5 atomes, de carbone, et Y et Y' sont indépendamment l'hydrogène, un groupe NH<sub>4</sub> ou un métal alcalin.

13. Composition suivant la revendication 9, qui comprend en outre un réactif produisant une coloration.

14. Composition suivant la revendication 9, dans laquelle l'acide aminé ou un de ses sels est le glutamate de sodium.

15. Composition suivant la revendication 9, dans laquelle l'acide aminé ou un de ses sels est l'aspartate de sodium.

16. Trousse d'essai diagnostique pour la détermination du peroxyde d'hydrogène, qui comprend

a) un récipient ayant une composition comprenant:

— une solution tampon,

— une oxydase choisie parmi la glycérol-3-phosphate oxydase, la choline oxydase, et la glucose oxydase, et

— un acide aminé ou un de ses sels, et

b) un récipient ayant une composition comprenant:

— un réactif produisant une coloration, et

— un solvant pour le réactif produisant une coloration.

17. Utilisation d'une composition d'oxydase stabilisée par un acide aminé, comprenant:

— de la glycérol-3-phosphate oxydase,

— une solution tampon,

— un acide aminé ou un de ses sels,

— de la peroxydase, et

— de la 4-aminoantipyrine,

pour la détermination quantitative de la teneur en glycérol-3-phosphate dans un fluide biologique.

18. Utilisation suivant la revendication 17, dans laquelle l'acide aminé ou un de ses sels est un sel de métal alcalin d'un acide aminé.

19. Utilisation suivant la revendication 18, dans laquelle le sel de métal alcalin d'un acide aminé est l'aspartate de sodium ou le glutamate de sodium.

20. Utilisation d'une composition d'oxydase stabilisée par un acide aminé, comprenant

— une lipase,

— de la glycérol kinase,

— de la glycérol-3-phosphate oxydase,

— de la peroxydase,

— de la 4-aminoantipyrine,

— de l'adénosine triphosphate,

— un acide aminé ou un de ses sels, et

— une solution tampon,

pour la détermination quantitative de la teneur en triglycérides d'un fluide biologique.

21. Utilisation suivant la revendication 20, dans laquelle l'acide aminé ou un de ses sels est un sel de métal alcalin d'un acide aminé.

22. Utilisation suivant la revendication 21, dans laquelle le sel de métal alcalin d'un acide aminé est l'aspartate de sodium ou le glutamate de sodium.

23. Utilisation d'une composition d'oxydase stabilisée par un acide aminé, comprenant

— de la choline oxydase,

— de la peroxydase,

— de la 4-aminoantipyrine,

— un acide aminé ou un de ses sels, et

— une solution tampon,

pour la détermination quantitative de la teneur en choline dans un fluide biologique.

24. Utilisation suivant la revendication 23, dans laquelle l'acide aminé ou un de ses sels est un sel de métal alcalin d'un acide aminé.

25. Utilisation suivant la revendication 24, dans laquelle le sel de métal alcalin d'un acide aminé est l'aspartate de sodium ou le glutamate de sodium.

26. Utilisation d'une composition d'oxydase stabilisée par un acide aminé, comprenant:

— de la choline oxydase,

— de la peroxydase,

— de la 4-aminoantipyrine,

— du chlorure de choline benzoyle,

— un acide aminé ou un de ses sels, et

— une solution tampon,

pour mesurer l'activité de la choline estérase dans un fluide biologique.

27. Utilisation suivant la revendication 26, dans laquelle l'acide aminé ou un de ses sels est un sel de métal alcalin d'un acide aminé.

28. Utilisation suivant la revendication 27, dans laquelle le sel de métal alcalin d'un acide aminé est l'aspartate de sodium ou le glutamate de sodium.

29. Utilisation d'une composition d'oxydase stabilisée par un acide aminé, comprenant:

— de la glucose oxydase,

— de la peroxydase,

— de la mutarotase,

— de la 4-aminoantipyrine,

— un acide aminé ou un de ses sels, et

— une solution tampon,

pour la détermination quantitative de la teneur en glucose dans un fluide biologique.

30. Utilisation suivant la revendication 29, dans laquelle l'acide aminé ou un de ses sels est un sel de métal alcalin d'un acide aminé.

31. Utilisation suivant la revendication 30, dans laquelle le sel de métal alcalin d'un acide aminé est l'aspartate de sodium ou le glutamate de sodium.

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60

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FIG. 1

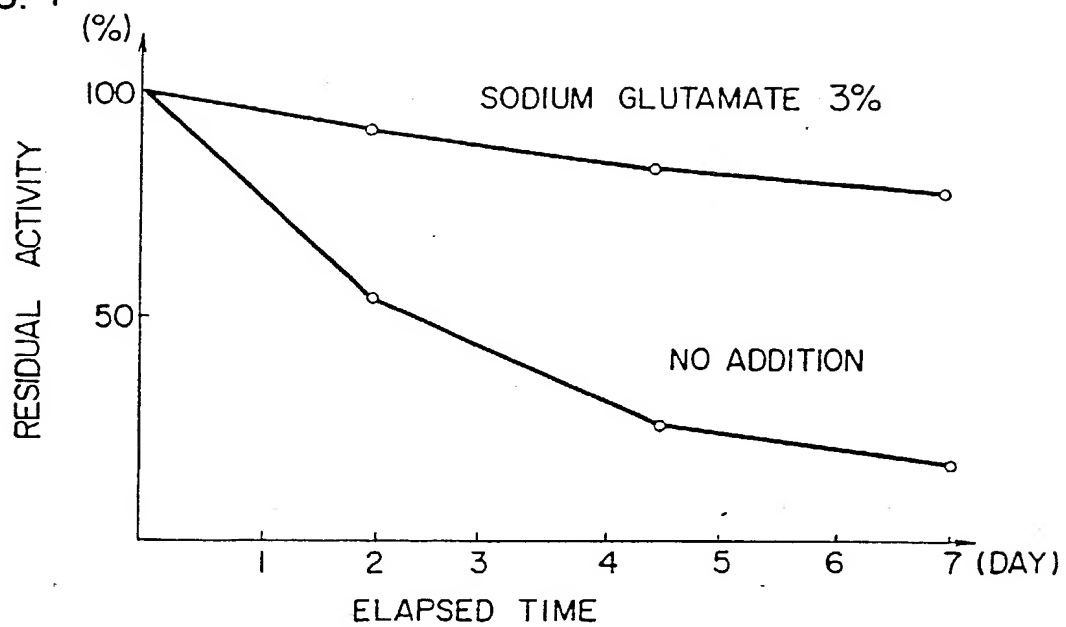


FIG. 2

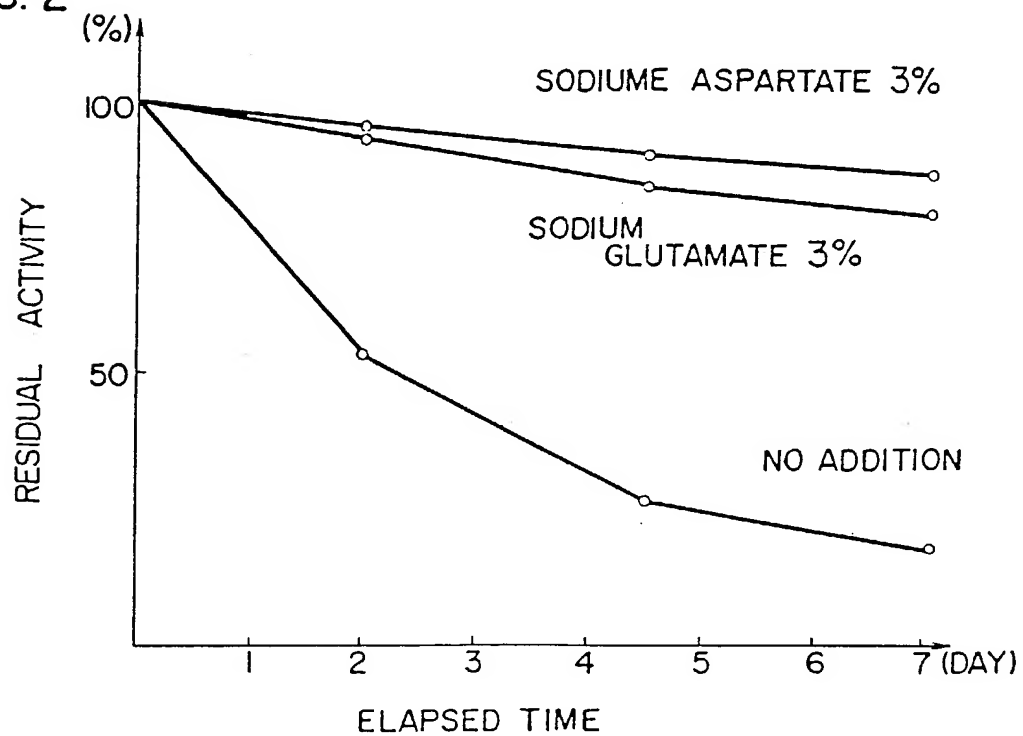


FIG. 3

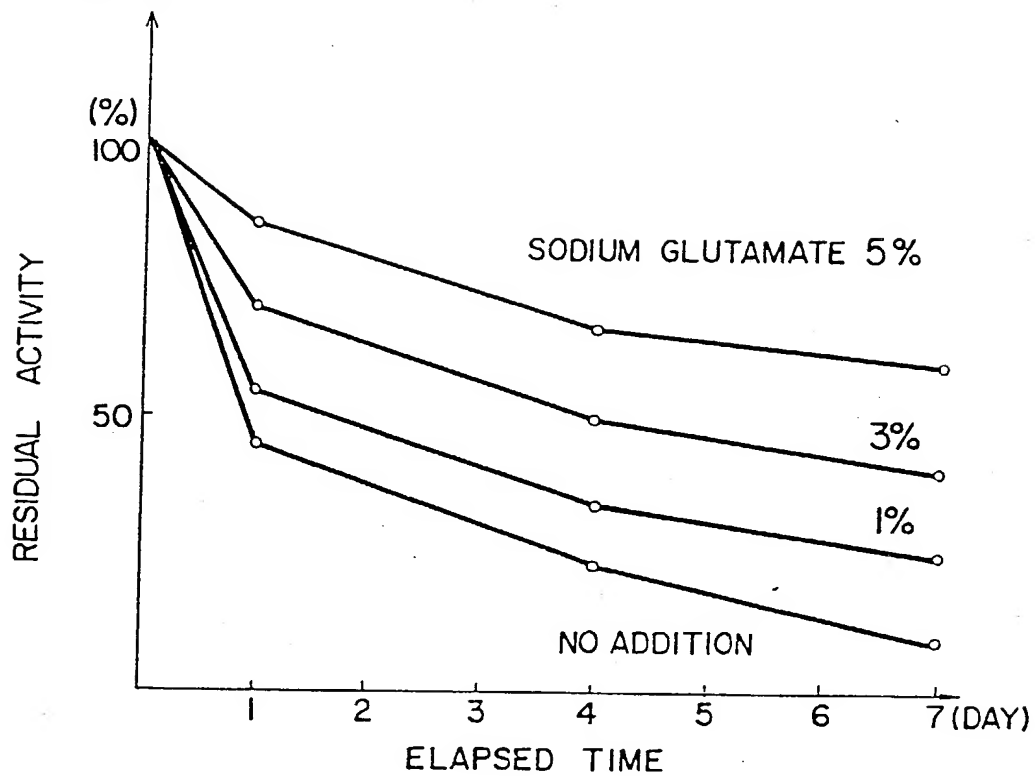


FIG. 4

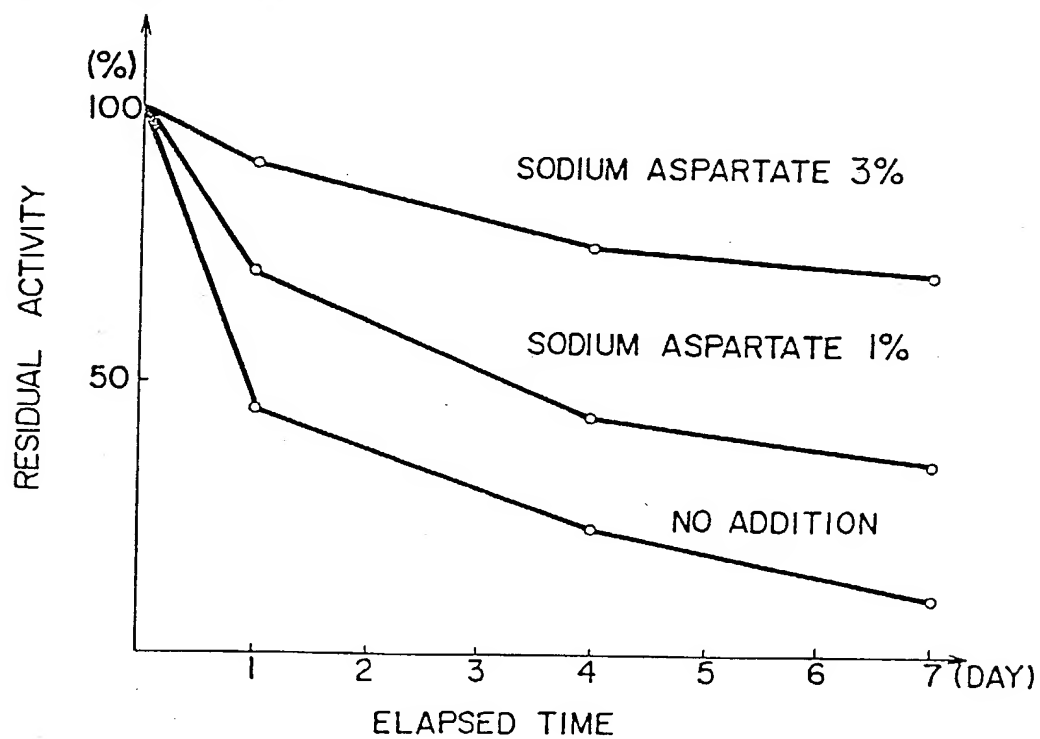


FIG. 5

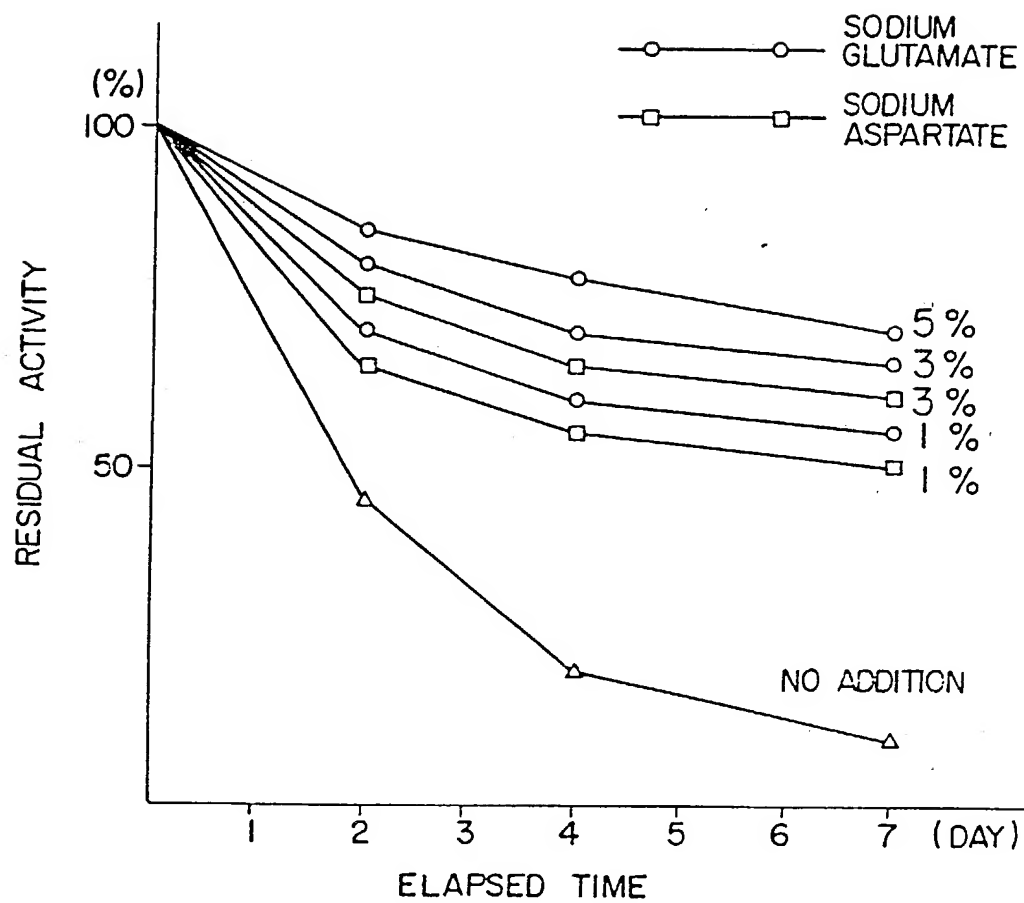




FIG. 6

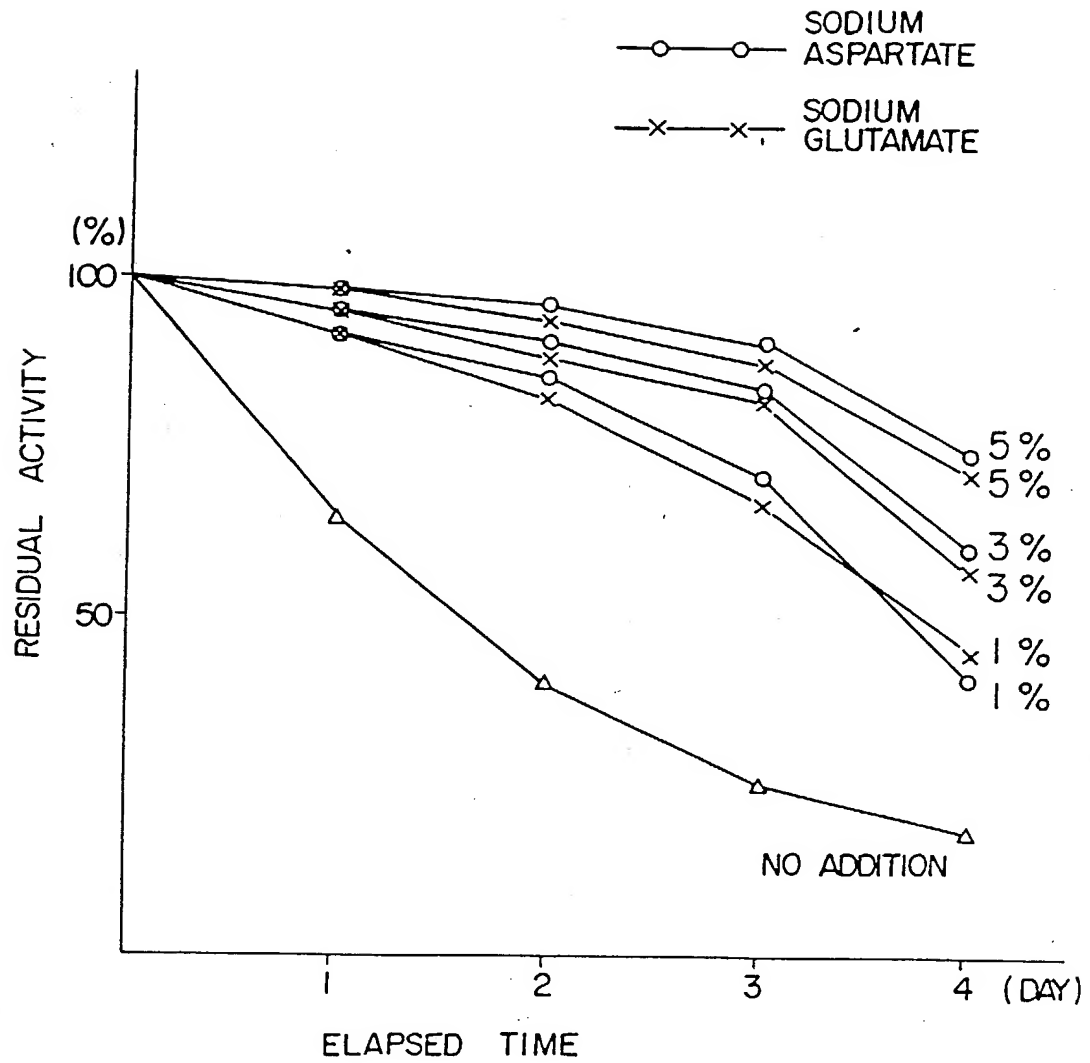


FIG. 7

